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# Effective vaccination against rabies in puppies in rabies endemic regions

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**In rabies endemic regions, a proportionally higher incidence of rabies is often reported in dogs younger than 12 months of age, which includes puppies less than 3 months of age; this presents a serious risk to public health. The higher incidence of rabies in young dogs may be the effect of low vaccination coverage in this age class, partly as a result of the perception that immature immune systems and maternal antibodies inhibit seroconversion to rabies vaccine in puppies less than three months of age. Therefore, to test this perception, the authors report the virus neutralising antibody titres from 27 dogs that were vaccinated with high quality, inactivated rabies vaccine aged three months of age and under as part of larger serological studies undertaken in Gauteng Province, South Africa, and the Serengeti District, Tanzania. All of these dogs seroconverted to a single dose of vaccine with no adverse reactions reported and with postvaccinal peak titres ranging from 2.0 IU/ml to 90.5 IU/ml. In light of these results, and the risk of human beings contracting rabies from close contact with puppies, the authors recommend that all dogs in rabies endemic regions, including those less than three months of age, are vaccinated with high quality, inactivated vaccine.**

## Introduction

Canine-mediated human rabies kills approximately 60,000 people every year (WHO 2013, Hampson and others 2015). Mortality from rabies is highest in less developed communities in Asia and Africa where domestic dogs are free-roaming (Ezeokoli and Umoh 1987, Butler and Bingham 2000, Kitala and others 2002, Kayali and others 2003, Windiyarningsih and others 2004, Reece and Chawla 2006, Kasempimolporn and others 2008, WHO 2013); with increasing evidence that the majority are owned (Ezeokoli and Umoh 1987, Butler and Bingham 2000, Estrada and others 2001, Windiyarningsih and others 2004, van Sittert and others 2010, Gsell and others 2012, Morders and others 2014b) and, thus, generally accessible for vaccination (Lembo and others 2010, Knobel and others 2013).

Mass vaccination of domestic dogs is key to the successful control of canine rabies, and a strong body of theoretical and empirical evidence indicates that vaccinating 70 per cent of the dog population during annual campaigns should be sufficient to control rabies (Coleman and Dye 1996, Cleaveland and others 2003, 2006, Belotto and others 2005, Schneider and others 2005, Hampson and others 2009, WHO 2013). Achieving vaccination coverage of 70 per cent during campaigns should maintain population immunity above the critical levels of 20–45 per cent required to interrupt rabies transmission (Coleman and Dye 1996, Hampson and others 2009). Effective coverage has been achieved through vaccinating juveniles and adults (Chomel and others 1987, Beran 1991, de Balogh and others 1993, Mitmoonpitak and others 1998, Matter and others 2000, Flores-Ibarra and Estrella-Valenzuela 2004, Touihri and others 2011), given that puppies less than three months of age are often excluded from vaccination programmes (Chomel and others 1987, Beran and Frith 1988, Brooks 1990, Matter and Fico 1998, Mitmoonpitak and others 1998, Matter and others 2000, Gunatilake and others 2003, Flores-Ibarra and Estrella-Valenzuela 2004, Awoyomi and others 2007, Durr and others 2009, Touihri and others 2011).

Low vaccination coverage in puppies has important implications for public health, especially as vaccination coverage of the population and, thus, herd immunity declines following a vaccination campaign. A proportionally higher incidence of rabies is often reported in dogs under 12 months of age, which includes puppies less than three months of age (Belcher and others 1976, Malaga and others 1979, Beran 1991, Mitmoonpitak and others 1998, Widdowson and others 2002). In these studies, the proportion of laboratory confirmed cases in dogs under three months of age range from 7.6 per cent to 17.4 per cent. This presents a serious risk to the public, given that the fraction of puppies less than three months of age in a population may be large, reportedly ranging from 4.1 per cent to 39 per cent (Davlin and

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VonVille 2012), and the close relationship between human beings and puppies (Mitmoonpitak and others 1997, Taiwo and others 1998, WHO 1998, Widdowson and others 2002, Awoyomi and others 2007).

Puppies less than three months of age are generally excluded from rabies vaccination programmes on the assumption that they have immature immune systems and maternal antibodies (Day 2007, Siegrist 2008, Hodgins and Shewen 2012) which may limit the immune response to rabies vaccine. Primarily to safeguard against possible inhibitory effects of maternal antibody, most manufacturers of high quality, inactivated rabies vaccines for dogs recommend a primary or booster vaccination at 12–13 weeks (Merial Animal Health Limited, MSD Animal Health). Similarly, internationally recognised vaccination guidelines for dogs recommend primary vaccination against rabies at 12–13 weeks of age (WSAVA 2010, AAHA 2011). Consequently, those administering vaccine under field conditions may be reluctant to use rabies vaccines off-label (Awoyomi and others 2007, Touihri and others 2011); even though World Health Organization (WHO) guidelines recommend that all dogs, including puppies less than three months of age, are vaccinated during mass vaccination campaigns (WHO 2004, 2013) when booster vaccinations are generally not available. Furthermore, owners also often perceive puppies as too young for vaccination (Flores-Ibarra and Estrella-Valenzuela 2004, Kongkaew and others 2004, Kaare and others 2009, Davlin and others 2013) so they are often not presented for vaccination during campaigns.

Evaluation of the effect of maternal antibodies and immune function of puppies on rabies vaccine induced immune responses is limited. Maternal antibody may interfere with immune responses (Day and Schultz 2011, Siegrist 2012, Tizard 2013), particularly in puppies eight weeks of age or younger vaccinated with modified live rabies vaccine under field conditions (Aghomo and others 1990). However, at least under experimental conditions, maternal antibodies and immune function may not limit the immune response to inactivated vaccines which stimulate both B cell and T cell responses (Siegrist 2012), as demonstrated in puppies vaccinated with Rabisin<sup>®</sup> (Merial Animal Health Limited) at two weeks of age (Chappius 1998). The authors present serological data from puppies vaccinated under field conditions in South Africa and Tanzania that support these prior observations.

## Materials and methods

Puppies (hereafter defined as dogs three months of age and under) were vaccinated as part of larger serological studies in five low-income communities of Africa where the dogs are owned, with the majority being mixed-breed and free to roam. The five communities include the township of Zenzele in Gauteng Province, South Africa (Morters and others 2014a, b), and four villages (Ngarawani, Rung'abure, Nyamburi and Bisarara, hereafter referred to as 'Serengeti') in the Serengeti District, Tanzania (McNabb 2008). Vaccinations for this study were undertaken during February 2010 in Zenzele and May 2008 in the Serengeti. None of the puppies were vaccinated prior to this study. Central point vaccination campaigns had also been undertaken in Zenzele by the Department of Agriculture (DoA) in May 2006. In the Serengeti, annual central point vaccinations have been undertaken since 2003 as part of studies to investigate and prevent canid diseases (Kaare and others 2009).

For the puppies in the Serengeti and those acquired from outside of Zenzele, age was reported by the owner but validated by direct observation and tooth eruption (Dyce and others 1987). For puppies born in Zenzele, age was determined from intensive monitoring of the dams generating reliable whelping dates, direct observation and tooth eruption (Morters and others 2014b).

In Zenzele, every available dog (n=259) in the entire population (of 315), including 68 puppies (from a total of 86 in the population) and their dams, were vaccinated door-to-door with

1 ml of Rabisin<sup>®</sup> (Merial Animal Health Limited), an inactivated rabies vaccine containing at least 1 International Unit (IU) of rabies virus glycoprotein GS57 Wistar strain. Vaccine was administered subcutaneously into the nape of the neck.

In the Serengeti, eight puppies in a convenience sample of 200 dogs brought to a central vaccination station were vaccinated with 1 ml of Nobivac<sup>®</sup> Rabies (MSD Animal Health) subcutaneously into the nape of the neck. Nobivac<sup>®</sup> Rabies contains >2 IU inactivated Rabies Virus strain Pasteur RIV. Ten ml of Nobivac<sup>®</sup> Rabies was used to reconstitute one vial of Nobivac<sup>®</sup> Puppy DP (containing live attenuated CPV strain C 154 and CDV strain Onderstepoort) (MSD Animal Health), thus the puppies were simultaneously vaccinated against Canine Parvo Virus (CPV) and Canine Distemper Virus (CDV). In addition, 0.01 ml/kg of ivermectin (Ivomec) was administered to a proportion of the puppies. In both locations the vaccine cold chain was carefully maintained.

The majority of the (68) puppies in Zenzele were <6–8 weeks of age when vaccinated and deemed too small to blood sample immediately prior to vaccination without causing unnecessary distress to the puppy and/or owner (see online supplementary Table S1 for the age distribution at vaccination of the (68) puppies). Therefore, prevaccinal virus neutralising antibody titres (hereafter referred to as 'titres') were obtained from only four of the puppies. To measure postvaccinal peak titres blood samples were collected approximately 30 days following vaccination. Thirty-seven of the 68 vaccinated puppies remained in Zenzele 30 days after vaccination, and of these 19 were big enough to blood sample (see online supplementary Table S1 for the outcomes of the (68) vaccinated puppies). In the Serengeti, blood samples were collected from all eight puppies immediately prior to vaccination and 21 days later. All samples were centrifuged within eight hours of collection, and the sera were either chilled or frozen from the time of collection until they were shipped to the Animal and Plant Health Agency, UK, where titres were measured by fluorescent antibody virus neutralisation (FAVN) test, a method prescribed by the Office International des Epizooties (OIE) (Cliquet and others 1998). Aliquots of the sera were also transported chilled from the Serengeti to Cornell University, USA, where titres for CDV and CPV were measured by virus neutralisation and haemagglutination inhibition tests, respectively.

All puppies were examined by a veterinarian at the time of vaccination and blood sampling. In Zenzele, every owner was made aware of the emergency phone number (written on their dog's vaccination certificate) to contact the veterinarian if any abnormalities in the health or behaviour of their dog were observed following vaccination. Every house in Zenzele in which a puppy was vaccinated in February 2010 was revisited twice by the veterinarian during March 2010 to collect (i) demographic data by direct observation and owner questionnaire, and (ii) day 30 postvaccinal blood samples as part of larger dog demography (Morters and others 2014b) and aforementioned serological (Morters and others 2014a) studies, respectively. During each visit, every available vaccinated puppy underwent a health assessment irrespective of whether a blood sample was collected or not. See online supplementary Table S1 for a description of the health assessments of the (68) puppies vaccinated in Zenzele in February 2010.

The study in South Africa was approved by the Ethics Committee, University of Cambridge, and the Research and Animal Ethics Committees, University of Pretoria, and the study in Tanzania was approved by the Tanzanian Commission for Science and Technology, Tanzania Wildlife Research Institute, and the Royal (Dick) School of Veterinary Studies, Edinburgh. In Tanzania the blood samples were collected during an ongoing vaccination programme undertaken by the Serengeti Health Initiative. In all cases, vaccination and blood sampling were only carried out with the owner, or responsible adult delegated by the owner, present and their informed consent.

## Results

In Zenzele, titres for the four puppies sampled immediately prior to vaccination were  $\leq 0.13$  IU/ml, similar to prevaccinal titres in 32 dogs 1.5–4.5 months of age from dams vaccinated with high quality, inactivated vaccine against rabies in Thailand (Kasempimolporn and others 1996, Tepsumethanon, personal communication, 2015). Prevaccinal titres for the eight puppies in the Serengeti were  $< 0.3$  IU/ml (ranging from 0.07 IU/ml to 0.29 IU/ml). Postvaccinal peak (i.e. day 30) titres for the (19) puppies in Zenzele are shown in Table 1. All of the puppies seroconverted to the vaccine (i.e. generated titres  $\geq 0.5$  IU/ml (Kennedy 1998)), with a geometric mean titre of 20.7 IU/ml.

Seventeen of the 19 puppies (blood sampled on day 30) were born in Zenzele to eight dams; all of the adult females were seronegative ( $< 0.5$  IU/ml) immediately prior to vaccination in February 2010 (Table 1). Five of the dams were present in Zenzele in May 2006 and may have been vaccinated by the DoA, however none had a titre suggestive of an anamnestic response to vaccination (defined as a peak titre  $\geq 128$  IU/ml (Morters and others 2014a)) in February 2010 (day 30 titres ranged from 0.09 IU/ml to 90.5 IU/ml). The other two puppies (blood sampled on day 30) were obtained from outside Zenzele, therefore the vaccination status of their dams was not known. Only 5 of the (68) vaccinated puppies were still in Zenzele 90 days after vaccination, and of these 4 remained 12 months after vaccination (with day 360 titres of 0.09 IU/ml, 0.35 IU/ml, 0.35 IU/ml and 1 IU/ml). This includes one puppy born in Zenzele that was not blood sampled 30 days following vaccination; however titres for this puppy 90 days, 180 days and 360 days following vaccination were  $\geq 0.5$  IU/ml. The dam of this puppy was vaccinated with Rabisin<sup>®</sup> in October 2009 with an anamnestic response to the vaccine (day 30 titre of 128 IU/ml), consistent with possible vaccination also in May 2006. See online supplementary Table S1 for the prevaccinal and postvaccinal titres of the (68) puppies and their dams.

All (8) puppies in the Serengeti seroconverted to the rabies vaccine at 21 days following vaccination, with all titres exceeding 5.9 IU/ml (Table 2). In this site, no data were available for end-point titres, vaccination status of the dams or survival of the puppies beyond the 21-day follow-up period. In the Serengeti, where CDV and CPV were administered simultaneously with Nobivac<sup>®</sup> Rabies, all (8) puppies also seroconverted to CDV and CPV with high 21-day postvaccinal antibody titres of  $\geq 256$  and  $\geq 640$ , respectively. See online supplementary Table S2 for the prevaccinal and postvaccinal CDV and CPV titres.

TABLE 1: Day 0 (prevaccination) and day 30 (peak) titres of the puppies vaccinated in Zenzele

Dog	Gender	Age at vaccination (weeks)	Puppy day 0 titres (IU/ml)	Puppy day 30 titres (IU/ml)	Dam day 0 titres (IU/ml)	Dam present May 2006
1	f	8–10	0.06	11.3	0.06	Yes
2	m	8–10	0.06	2.0	0.06	Yes
3	f	7–8	–	45.3	0.18	Yes
4	m	6–7	–	22.6	0.06	No
5	m	6–7	–	45.3	0.06	No
6	f	7–8	–	16.0	0.06	No
7	m	5–6	–	64.0	0.06	No
8	f	4–6	–	45.3	0.06	Yes
9	f	5–7	–	32.0	0.06	Yes
10	f	5–7	–	64.0	0.06	Yes
11	m	5–7	–	5.7	0.06	Yes
12	m	4–6	–	45.3	0.18	Yes
13	f	4–6	–	90.5	0.18	Yes
14	f	5	–	22.6	0.06	Yes
15	f	5	–	8.0	0.06	Yes
16	f	5	–	5.7	0.06	Yes
17	f	10 days	–	5.7	0.09	No
18	f	6–8	–	32.0	–	–
19	f	10–12	–	22.6	–	–

f, female; m, male

At the time of blood sampling and the health assessment, there were no reports or clinical signs of type IV hypersensitivity reactions (i.e. granulomas or sterile abscesses at the injection site), the main risk associated with the use of inactivated vaccine with adjuvant (Merial Animal Health Limited, MSD Animal Health, Tizard 2013). Although 14 puppies in Zenzele died before the first household visit in March 2010 (see online supplementary Table S1), none were reported to have died the day of vaccination, suggestive of a type I hypersensitivity (anaphylactoid) reaction which may occur up to two hours or three hours following vaccination (Tizard 2013); nor were there any reports of type IV hypersensitivity reactions.

## Discussion

The present study shows that puppies from low-income communities in rabies endemic regions respond well to a standard dose of high quality, inactivated rabies vaccine without any apparent adverse reactions (Merial Animal Health Limited, MSD Animal Health, Tizard 2013). All the puppies sampled following vaccination in this study generated antibody titres  $> 0.5$  IU/ml after vaccination, and most individuals recorded much higher titres. Although the sample was small, somewhat related to the poor general background survival of puppies here (see online supplementary Table S1), this result was consistent across the study group irrespective of the levels of prevaccinal antibody, the administration of ivermectin at the time of vaccination, or concurrent vaccination against CDV and CPV. Nonetheless, given the lack of published data, larger field studies to investigate the effects of ivermectin on immunological responses to inactivated rabies vaccine may be warranted given that ivermectin is often administered as part of rabies vaccination programmes.

None of the puppies had prevaccination antibody titres  $> 0.5$  IU/ml that might be indicative of maternal antibody against rabies. However, because of the uncertain vaccination status of the dams, it was not possible to determine whether the low prevaccinal antibody titre of 0.29 IU/ml detected in one puppy in the Serengeti was the result of maternal antibody. More detailed studies of the maternal antibody status and immunological responses of puppies in these low-income settings is also warranted, particularly as the development of large-scale rabies control and elimination programmes across Asia and Africa (Lapiz and others 2012, Putra and others 2013, WHO 2013) is likely to result in an increasing proportion of puppies born to vaccinated dams.

Rabies is a serious zoonosis that remains uncontrolled in dog populations throughout much of Asia and Africa. Given the inadequacy of vaccination campaigns, a substantial proportion of free-roaming dogs in affected communities are never vaccinated or vaccinated only once in their lifetime (Mitmoonpitak and others 1998, Lembo and others 2010). Although mortality in puppies less than three months of age is generally high in these populations (Brooks 1990, de Balogh and others 1993, Kitala and others 2001, Gsell and others 2012, Morters and others 2014b), delaying vaccination until puppies are three months of age may result in these

TABLE 2: Day 0 (prevaccination) and day 21 (peak) titres of the puppies vaccinated in the Serengeti

Dog	Gender	Age at vaccination (months)	Day 0 titres (IU/ml)	Day 21 titres (IU/ml)	Ivermectin administered
1	m	3	0.17	$> 5.9$	No
2	m	3	0.07	$> 5.9$	Yes
3	f	2	0.29	$> 5.9$	Yes
4	f	3	0.07	$> 5.9$	No
5	m	3	0.07	$> 5.9$	Yes
6	m	3	0.07	$> 5.9$	Yes
7	f	2	0.07	$> 5.9$	Yes
8	f	3	0.17	$> 5.9$	Yes

f, female; m, male

dogs never being vaccinated. On the basis of results of the present study, and the risk of human beings contracting rabies from young puppies (Mitmoonpitak and others 1997, Taiwo and others 1998, WHO 1998, Widdowson and others 2002, Awoyomi and others 2007), all dogs in rabies endemic regions, including puppies less than three months of age, should be vaccinated against rabies as recommended by the WHO (WHO 2004, 2013). While puppy vaccination should therefore be included in annual rabies vaccination campaigns, these efforts should not compromise vaccination of juvenile and adult dogs, which have higher survival rates than puppies and are therefore important in maintaining vaccination coverage between campaigns (Morters and others 2014b). As humoral immunity can wane rapidly in young dogs (Siegrist 2012) and puppies are continually acquired by community members throughout the year, it is recommended that all young dogs should also be given primary and booster vaccinations whenever veterinary services are available to dog owners.

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